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PERMANENT GENETIC RESOURCES NOTE

Nineteen novel microsatellite markers for the Olympia oyster, *Ostrea conchaphila/lurida*

DAVID A. STICK,*‡ CHRIS J. LANGDON,*‡ MICHAEL A. BANKS*‡ and MARK D. CAMARA†‡
*Department of Fisheries and Wildlife, Coastal Oregon Marine Experiment Station, Oregon State University, †USDA-Agricultural Research Service, ‡Hatfield Marine Science Center, 2030 SE Marine Science Drive, Newport, OR 97365, USA

Abstract

Accurate evaluation of remnant *Ostrea conchaphila/lurida* population structure is critical for developing appropriate restoration efforts. Here we report 19 polymorphic microsatellites suitable for analyses of population differentiation, pedigree reconstruction and linkage map construction. We screened clones from four enriched genomic libraries, identified 73 microsatellite-containing sequences and designed polymerase chain reaction primers for 44 of these loci. We successfully optimized polymerase chain reaction conditions for 20 loci, including one monomorphic locus. In a Willapa Bay reference sample, mean observed and expected heterozygosities were 0.6729 and 0.8377. Nine loci deviated from Hardy-Weinberg equilibrium. These markers have proven useful for genetic studies of the Olympia oyster.

Keywords: microsatellites, Olympia oyster, *Ostrea conchaphila/lurida*, PCR

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The Olympia oyster, *Ostrea conchaphila/lurida* Carpenter 1857/1864, is the only oyster species native to the US Pacific Northwest. Although controversy surrounds the correct nomenclature of this species, recent genetic evidence supports Carpenter's original classification of northern populations as *O. lurida* and populations from mainland Mexico as *O. conchaphila* (Polson *et al*. 2008). Historically, this species ranged from southeastern Alaska southward through Mexico in densities capable of supporting both tribal subsistence fisheries and large commercial harvests. Overexploitation, habitat degradation, competition and predation from non-native species have drastically depleted or extirpated many local populations. Ecological benefits provided by oyster reef habitats and the species' historical significance has fueled numerous restoration and supplementation efforts. Unfortunately, these efforts are proceeding without a clear understanding of existing genetic structure among populations, which could be substantial as a consequence of limited dispersal and/or anthropogenic impacts such as genetic bottlenecks or population admixture. Microsatellites developed for other oyster species, specifically *Crassostrea gigas* and *Ostrea edulis*, failed to amplify in *O. conchaphila/lurida*. Here we report 19 novel microsatellite primer sets designed specifically for this species.

Correspondence: David A. Stick, Fax: 541-867-0345; E-mail: david.stick@oregonstate.edu

We extracted high molecular weight DNA from the adductor muscle and mantle of a Willapa Bay, Washington oyster using the DNeasy Tissue Kit (QIAGEN Inc.) and further concentrated it as described in Sambrook *et al*. (1989). Genetic Identification Services constructed four genomic DNA libraries enriched for repeated CA, AAT, ATG, TAGA motifs based on methodology described in Jones *et al*. (2002) and sequenced randomly selected clones from all four libraries on an ABI PRISM 377 DNA sequencer using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences). Polymerase chain reaction (PCR) primers were designed for the regions flanking confirmed microsatellite sequences using DesignerPCR version 1.03 (Research Genetics Inc.). We optimized PCR conditions using DNA similarly extracted from four Yaquina Bay, Oregon oysters. Optimized primers were evaluated using a reference population consisting of 100 individuals from Willapa Bay, Washington.

We performed 5 μL PCRs containing the following components: locus-specific ($MgCl_2$) (Table 1), 1× Go*Taq* FlexiPCR buffer, pH 8.5 (Promega), 0.15 mM dNTPs (Promega), 0.2 μM 5′-fluorescently labelled forward (ABI) and unlabelled reverse (Integrated DNA Technologies Inc.) primers (Table 1) and 0.025 U/μL Go*Taq* FlexiDNA

Table 1 Characterization of microsatellite loci for the Olympia oyster, *Ostrea conchaphila/lurida*. Locus names with * indicate Mendelian segregation tested and verified; θ indicate presence of null alleles as determined by Micro-Checker. Significant departures of observed heterozygozity from Hardy–Weinberg equilibrium (HWE) after sequential Bonferroni correction are in boldface type (least significant α after correction = 0.00417). Frequencies of null alleles estimated as the Brookfield 1 (B1) and Brookfield 2 (B2) null allele estimators using Micro-Checker

Locus	Clone ID	GenBank Accession no.	Primer sequences	5′ Fluorescent label	Repeat motif of clone	Annealing temp. (°C)	($MgCl_2$) (mM)	Allele size range (bp)	No. of alleles observed (N_A)	No. of samples amplified (n)	Frequency of null alleles (B1 / B2)	Expected heterozygosity (H_E)	Observed heterozygosity (H_O)	P value HWE test
*Olur01*θ	A102	EU587388	F: 5′-AAGACACTGTATAGCGGTAAGA-3′ R: 5′-AAGACCCGTGATTCTCAC-3′	VIC	$(GT)_{14}$	50	2.0	220–240	6	97	0.1755/0.2416	0.5659	**0.2887**	**< 0.0001**
*Olur02*θ	A103	EU587389	F: 5′-TGCGACAAATGCACGTAC-3′ R: 5′-GTCGGAAGACAGAGGCTACA-3′	FAM	$(GA)_9$	56	1.5	231–273	12	96	0.2005/0.2774	0.6189	**0.2917**	**< 0.0001**
*Olur03*θ	A103a	EU587390	F: 5′-TCTTCCGACAACGTACTATTT C-3′ R: 5′-CCGGTTTTTAAGGGTCATATA-3′	VIC	$(CA)_{12}$	50	2.0	212–261	16	85	0.3079/0.4887	0.8238	**0.2588**	**< 0.0001**
*Olur04*θ	A115	EU587391	F: 5′-GTTGGGAATGAGTTTCAAGGT-3′ R: 5′-TGCCTAGATTACCTCAAAATCC-3′	PET	$(GT)_{22}$	48	1.5	187–294	43	100	0.0201/0.0201	0.9643	0.9200	0.3404
*Olur05**θ	C2	EU587392	F: 5′-CAGCAGATGACAAGATAAGCTC-3′ R: 5′-CAGGTGTCGTCACTATTGTTG-3′	VIC	$(CAT)_{15}$	56	2.0	249–355	26	100	0.0671/0.0671	0.9019	**0.7700**	**0.0041**
*Olur06**	C4	EU587393	F: 5′-CCATCCTGTGTTTCAAATTCC-3′ R: 5′-CAAGGCTTATCTTTCCTGGTG-3′	NED	$(CAT)_2$ $CGT(CAT)_5$	60	3.0	233	1	100	0.0/0.3317	0.8900	0.0000	n/a
*Olur07**θ	C6	EU587394	F: 5′-ACATGCTAACAAGATTCAGATC-3′ R: 5′-ATCAGATGATGACGATGTATTG-3′	FAM	$(CAT)_{18}$	56	1.5	168–258	21	99	0.1162/0.1438	0.6606	**0.4444**	**< 0.0001**
*Olur08**θ	C7	EU587395	F: 5′-CGAATCGAATCAGTTGAAATAC-3′ R: 5′-AAATGATGATGGACACTGGTAG-3′	VIC	$(ATG)_{10}$	54	3.0	199–338	24	88	0.0882/0.2820	0.8243	**0.6591**	**< 0.0001**
*Olur09**θ	C9	EU587396	F: 5′-ATCTCCAGTTAAATCCCCATAC-3′ R: 5′-CGTCCTCAGATGATGATTATTC-3′	NED	$(CAT)_7$	54	1.5	195–213	7	99	0.1497/0.1713	0.7738	**0.5051**	**< 0.0001**
Olur10	C105	EU587397	F: 5′-TGCTTCAGTCACTTATCAACAG-3′ R: 5′-AGGAGGAGTAGCATTCCTTG-3′	NED	$(CAT)_{11}$	56	1.5	214–312	24	100	0.001/0.001	0.9164	0.9100	0.5450
Olur11	C122	EU587398	F: 5′-CTCGCCATCACTTACACTTC-3′ R: 5′-TGGAGAGCAAAACGATTATG-3′	NED	$(CAT)_{14}$	56	2.5	137–180	10	100	–0.0122/0.0	0.6036	0.6200	0.1059
Olur12	C123	EU587399	F: 5′-CATGCGGACAAAACTTTG-3′ R: 5′-CAGAAGCTGGTCAACTGATC-3′	FAM	$(CAT)_{11}$	56	2.0	180–275	16	100	–0.0206/0.0	0.7281	0.7600	0.7793
*Olur13**	D3	EU587400	F: 5′-GTGAAACATTCTTTCCTGAGTG-3′ R: 5′-CGAGTTCGACATAATGAAGTTC-3′	PET	$(ATCT)_{17}$	52	2.0	230–314	21	95	0.0051/0.0955	0.9306	0.9158	0.2792
*Olur14**θ	D6	EU587401	F: 5′-TGACCAAAAAACAGCTACTTCTG-3′ R: 5′-ACATGCCGTTACTCCTCTG-3′	VIC	$(GATA)_{18}$ $AATA(GATA)_4$	50	2.5	230–366	34	94	0.0491/0.1462	0.9630	**0.8617**	**< 0.0001**
*Olur15**	D8	EU587402	F: 5′-CTTTCCATCGAGTTCGACATAA-3′ R: 5′-GGTGCGGACTGTGATGTAATAC-3′	PET	$(TAGA)_{12}$	56	1.5	150–233	22	100	–0.0169/0.0	0.9322	0.9600	0.5412
*Olur16**θ	D12	EU587403	F: 5′-AGCATCGAACAAGCACTAAA-3′ R: 5′-GGAATTGAAACTCTCAAAGTTG-3′	FAM	$(GATA)_{21}$	50	1.5	236–451	32	97	0.1777/0.2264	0.9356	**0.5876**	**< 0.0001**
Olur17	D101	EU587404	F: 5′-ATCGAAACTGAACGAGTGTTG-3′ R: 5′-TTGGTCACTGATTGCTGAAAC-3′	FAM	$(TCTA)_{24}$ $TC(CATC)_9$	60	3.0	196–292	27	100	–0.0098/0.0	0.9258	0.9400	0.0211
*Olur18*θ	D104	EU587405	F: 5′-TGGTGTCCTTTATATCGAGTTC-3′ R: 5′-CGCTATTTGTGGGGAGAT-3′	PET	$(TATC)_{21}$ $TGTC(TATC)_3$	56	2.0	211–357	29	99	0.0275/0.0513	0.9367	0.8788	0.1204
Olur19	D107	EU587406	F: 5′-CTTTCCATCGAGTTCGACATAA-3′ R: 5′-TTAGCGTGTAGTCAACGGTCTC-3′	PET	$(GATA)_{20}$	54	2.5	202–285	21	100	–0.0170/0.0	0.9320	0.9600	0.5064
Olur20	D127	EU587407	F: 5′-TCCTTATGTTGGTCACTGATTG-3′ R: 5′-ATCGAAACTGAACGAGTGTTG-3′	NED	$(TGGA)_{12}$ $(TAGA)_{17}$	56	2.0	204–301	28	96	–0.0025/0.0752	0.9271	0.9271	0.0172

Polymerase (Promega) on an MJ Research PTC-225 Tetrad thermocycler running the following program: (i) denaturing for 5 min at 94 °C, (ii) amplification using 40 cycles of 30 s at 94 °C followed by 30 s at the locus-specific annealing temperature (Table 1) and 45 s at 72 °C, and (iii) final extension for 30 min at 72 °C. We resolved products using a 3730xl automated DNA sequencer with GeneScan 500 LIZ size standard (ABI) and scored them using GeneMapper version 3.7 (ABI). For Willapa Bay reference samples, we performed PCRs on a GeneAmp PCR 9700 thermocycler (ABI) using the same parameters. We also produced five full-sib Olympia oyster families at the Hatfield Marine Science Center and extracted DNA from each parental pair and 94 of their 14-day-old larvae. We verified Mendelian segregation using contingency table analysis against Mendelian expectations. We further tested observed heterozygosities for deviations from Hardy–Weinberg equilibrium and linkage disequilibrium using GenePop version 4.0.7 (Raymond & Rousset 1995). We estimated null allele frequency using Micro-Checker version 2.2.3 (van Oosterhout *et al*. 2004).

We found microsatellite-containing sequences in 73 of 100 clones screened. We designed primers for 44 sequences after excluding those that failed to meet default design parameters. Any marker that resulted in poor or no amplification, nonspecific amplification, multiple alleles or excessive null alleles was excluded from further development. We successfully optimized conditions for 20 primer pairs. Observed number of alleles per locus ranged from one to 43, with an average of 21 in the reference population (Table 1). The mono-allelic *Olur06* has been found to be polymorphic in other populations (D. A. Stick, unpublished data). Mean observed and expected heterozygosities were 0.6729 (± 0.2884) and 0.8377 (± 0.1319). After sequential Bonferroni correction, (Rice 1989), nine of the 19 tests for deviations from Hardy–Weinberg equilibrium (*Olur01*, *Olur02*, *Olur03*, *Olur05*, *Olur07*, *Olur08*, *Olur09*, *Olur14*, *Olur16*) and five out of 171 pairwise tests for linkage disequilibrium (*Olur02-Olur03*; *Olur13-Olur15*; *Olur10-Olur17*; *Olur13-Olur19*; *Olur15-Olur19*) were significant. Deviation from Hardy–Weinberg equilibrium is common in oyster species as a consequence of null alleles (Hedgecock *et al*. 2004). Null alleles are suspected to be segregating in all loci except *Olur06*, *Olur10*, *Olur11*, *Olur12*, *Olur13*, *Olur15*, *Olur17*, *Olur19* and *Olur20* (Table 1).

We confirmed Mendelian segregation for nine loci by comparing observed larval genotypic frequencies to expectations based on parental genotypes. We observed no cross-amplification in a panel of six individuals from each of the following species: *O. edulis, C. gigas, Crassostrea virginica, C. ariakensis, C. sikamea and C. hongkongensis*. We are currently using these loci in studies of population differentiation (D. A. Stick, in preparation). They should also be suitable for monitoring the effects of ongoing restoration efforts using parentage analysis and linkage map construction.

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References

Hedgecock D, Li G, Hubert S, Bucklin K, Ribes V (2004) Widespread null alleles and poor cross-species amplification of microsatellite DNA loci cloned from the Pacific oyster, *Crassostrea gigas*. *Journal of Shellfish Research*, **23**, 379–385.

Jones K, Levine K, Banks J (2002) Characterization of 11 polymorphic tetranucleotide microsatellites for forensic applications in California elk (*Cervus elaphus canadensis*). *Molecular Ecology Notes*, **2**(4), 425–427.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**(3), 535–538.

Polson MP, Hewson WE, Eernisse DJ, Baker PK, Zacherl DC (2008) You say *conchaphila*, I say *lurida*: resolving the taxonomic controversy of the Olympia oyster. *Journal of Shellfish Research* (in review).

Raymond M, Rousset F (1995) GenePop 4.0.7 (update of version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Sambrook J, Fritsh EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, New York.